ANTIOXIDANT AND ANTIFUNGAL ACTIVITIES

OF SOME MALAYSIAN COMMERCIAL TIMBERS

by

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ABBREVIATIONS

BuOH	Butanol
CC	Column chromatography
$CDCl_3$	Deuterated chloroform
CD_3COCD_3	Deuterated acetone
COSY	Correlated spectroscopy
DEPT	Distortionless enhancement by polarization transfer
DPPH	1,1'-diphenyl-2-picrylhydrazyl
EtOAc	Ethyl acetate
EtOH	Ethanol
g	Gram
H_2O_2	Hydrogen peroxide
HPLC	High performance liquid chromatography
MeOH	Methanol
ml	Mililiter
mm	Milimeter
Na_2CO_3	Sodium bicarbonate
NMR	Nuclear magnetic resonance
Py-GC/MS	Pyrolysis gas chromatography mass spectrometry
TLC	Thin layer chromatography
UV	Ultra-violet
v/v	Volume over volume
ORAC	Oxygen radical absorbance capacity

Aktiviti antioksidan dan antikulat beberapa spesies kayu dagangan Malaysia

ABSTRAK

Aktiviti antioksidan dan antikulat sebelas spesies kayu keras dagangan Malaysia daripada tujuh keluarga berlainan telah dikaji. Ekstrak metanol bahagian kulit, kayu teras dan kayu gubal daripada setiap spesies telah diuji menggunakan 1,1’-difenil-2-pikrilhidrazil (DPPH) untuk penentuan aktiviti antioksidan. Kandungan fenolik pula telah dinilai dengan menggunakan kaedah Folin-Ciocalteau. Ekstrak yang diuji daripada bahagian kulit Angsana, kulit dan kayu gubal Keruing latek, ketiga-tiga bahagian Kekatong, merpauh periang, kulit dan kayu gubal Bintangor bukit, dan kulit Sentul telah menunjukkan aktiviti antioksidan. Bahagian kulit Bintangor bukit telah dipecahkan lebih lanjut oleh kaedah kromatografi iaitu kolum kromatografi (CC) dan cecair-cecair (pembahagian) kromatografi (LLC). Catechol di samping beberapa sebatian lain seperti fenol, syringol, guaiacol dan 4-metilcatechol, telah dikenal pasti oleh pirolisis gas kromatogram jisim spektrometer (Py-GC/MS) sebagai sebatian yang paling penting bagi bahagian kulit Bintangor bukit untuk menyingkirkan radikal bebas.

Aktiviti antikulat ekstrak metanol bagi semua sampel telah dinilai menentang *Gloephyllum trabeum*, sejenis kulat perang dan *Pycnoporus sanguineus,* sejenis kulat putih. Kaedah antikulat menggunakan media di mana hifa kulat yang telah dihomogenesis disebarkan. Kajian menunjukkan hampir semua sampel mempamerkan reaksi antikulat sama ada menentang kulat perang atau kulat putih. Bahagian kayu teras Bintangor bukit telah menunjukkan aktiviti antikulat yang paling baik menentang kedua-dua kulat putih dan kulat perang. Sebatian yang memainkan peranan menyumbang kepada ciri antikulat Bintangor bukit daripada bahagian kayu teras telah disisihkan oleh

kaedah spektrometri HPLC dan penentuan struktur oleh ^{1}H NMR dan ^{13}C NMR telah mengenalpasti sebatian-sebatian 6-desoxyjacareubin dan 1,3,5-trihydroxy-2-3-(3-methylbut-2-enyl) xanthone.

Antioxidant and antifungal activities of some

Malaysian commercial timbers

ABSTRACT

Antioxidant and antifungal activities of eleven hardwood species of Malaysian commercial timbers from seven families were studied. Methanolic extracts from bark, heartwood and sapwood of each species were subjected to 1,1'-diphenyl-2-picrylhydrazyl (DPPH) for the antioxidant activity determination. Total phenolics were evaluated using the Folin-Ciocalteau method. The tested extracts of Angsana bark, Keruing latek bark and sapwood, all three parts of Kekatong, Merpauh periang bark, Bintangor bukit bark and sapwood, and Sentul bark did showed antioxidant activity. Bintangor bukit bark was further fractionated by column chromatography (CC) and liquid-liquid (partition) chromatography (LLC). Catechol with a few other compounds such as phenol, syringol, guaiacol and 4-methylcatechol were detected by pyrolysis gas chromatography mass spectrometry (Py-GC/MS) as the most important compounds responsible for Bintangor bukit bark to scavenge the free radicals.

The antifungal activity of methanolic extracts of all samples was evaluated against *Gloephyllum trabeum*, a brown rot fungus and *Pycnoporus sanguineu*s, a white rot fungus. The antifungal assay was done using a medium in which homogenized hyphae were dispersed. The investigation exhibited that most wood species showed antifungal activity against either brown rot or white rot fungus. Bintangor bukit heartwood showed the strongest antifungal activity against both brown rot and white rot fungus. The compound responsible for the antifungal property of Bintangor bukit heartwood were isolated by high performance liquid chromatography (HPLC), and identified by ^{1}H NMR

and ^{13}C NMR as 6-desoxyjacareubin and 1,3,5-trihydroxy-2-3-(3-methylbut-2-enyl) xanthone.

1.0 INTRODUCTION

1.1 Literature review

There are about 2500 different tree species grown in the tropical rainforest of Malaysia and 406 species are harvested for commercial use (MTC, 2004). Besides exporting timbers and timber products for the country earnings, various researches are continuously being done to maximize the potential of the woods to contribute in the field of science of natural sources.

The wood portion of a tree has two main parts. The outer, lighter coloured wood is the sapwood. It contains the living cells and takes part in the active life processes of the tree. Sapwood contains a lot of moisture, will shrink considerably when dried, and is much more susceptible to fungus. The inner, called heartwood consists of inactive cells and serves mainly to give strength to the tree. Heartwood starts forming in older growth rings near the pith. It is far less susceptible to fungus and doesn't contain nearly as much moisture as sapwood. There is little difference in the strength or physical characteristics of heartwood and sapwood from a given tree except for the slightly darker colour of heartwood. Whereas, surrounding the sapwood is the layer of bark. Bark is composed of tissues lying outside the cambium. (Thompson, 1960; Tsoumis, 1991).

The term extractives cover a large number of different compounds and can be extracted from wood by means of neutral organic solvents or water. The extractives are not part of the wood structure like cellulose and lignins, but they contribute such properties as colour, odour, taste and resistance to decay (Hillis, 1989). They include resins, fats, sugars, oils, starches, alkaloids, tannins, antioxidant compounds, etc. (Tsoumis, 1991; Kawamura et al., 2004; Lange and Hashim, 2001). Different types of extractives are necessary to maintain the diversified biological functions of a tree. Fats constitute the energy source of the wood cells, whereas, lower terpenoids, resin acids, and phenolic substances protect the wood against microbiological damage or insects attacks (Sjőstrőm, 1993).

Plants extracts have been shown to possess antioxidant activity (Furuta, et al., 1997; Sahu and Green, 1997; Cruz et al., 2005). Antioxidant is an inhibitor, which is effective in preventing oxidation by molecular oxygen and free radical reaction. Such inhibitors have great commercial significance in the foods and food products, medicines and pharmaceutical. Antioxidants are also used in the prevention of deterioration of petroleum products, rubber and plastics (Sahu and Green, 1997). Free radicals are present in biological systems from wide variety of sources and may oxidize nucleic acids, proteins, lipids or DNA. The 1,1'-diphenyl-2-picrylhydrazyl (DPPH) method is widely applied to evaluate the antioxidant activity (Cruz et al., 2005; Molyneux, 2004; Kitzberger et al., 2007). Stable free radical, DPPH·, accepts electron or hydrogen radical provided by antioxidant molecules and turns from purple to yellow coloured product to become a stable diagrammatic molecule resulting in a decrease in absorbance at 517 nm, read using UV/VIS spectrophotometer (Molyneux, 2004). Antioxidants may be water soluble, fat soluble, insoluble, or bound to cell and thus not necessarily freely available to react with DPPH, hence they react at different rates i.e. differing kinetics, and the reaction will often not completed in a reasonable assay time. Therefore, the sample size that can lower the initial absorbance of DPPH solution by 50% (EC_{50}) compared to that of a blank solution has been chosen as the endpoint for measuring the antioxidant activity (Molyneux, 2003; Kitzberger et al., 2007).

Phenolics are known to be the largest category of phytochemicals and the most widely distributed in the plant kingdom. The compounds showed one or more aromatic rings with one or more hydroxyl groups and are generally categorised as phenolic acids, stilbenes, flavonoids, coumarins and tannins. Generally, phenolics are the products of secondary metabolism in plants and provide essential functions in the production and growth of the plants (Ang, 2007). Phenolic compounds constitute the main class of natural antioxidants and are usually analyzed using the Folin-Ciocalteu method (Cruz et al., 2005). Due to their hydroxyl groups, phenols are known as one of the most important plant constituents because of their radical scavenging ability. The total phenolic content may contribute directly to the antioxidant action. Most of the works dealing natural products use gallic acid or catechin as standards which independently of phenolic species detected (Price et al., 1978; Duh et al., 1999). Phenolics also act as defense mechanisms against pathogens, insects, parasites, microbes, and predators, as well as contributing to the colour of the plants (Ang, 2007).

Plant pathogens, particularly fungi are responsible for yield reductions in food and crops throughout the world. Therefore, antifungal chemicals contribute substantially to the quality of food and human health by controlling many of the fungi that produce mycotoxins or by interfering in their biosynthesis (Carpinella et al., 2003). Despite the fact that many plants are affected by fungal disease, some of them are able to synthesize their own compounds (Quiroga et al., 2001; Carpinella et al., 2003; Kawamura et al., 2004; Kawamura and Ohara, 2005; Kusuma et al., 2005; Yen et al., 2007). These compounds can then become natural biodegradable pesticides or fungicides in the future.

Fungi are tiny, virtually microscopic, plants lacking chlorophyll and conductive tissues. Some of the fungi can grow and multiply only by living in a host plants for their whole life (obligate parasites). Some need a host plant for a part of their life cycles but can complete their cycles on artificial media, and there are others that can grow and multiply on dead organic matter as well as on living plants which also known as nonobligate parasites (Agrios, 1969). Diseases in plant caused by fungi are commonly by three major types of decay, namely white rot, brown rot and soft-rot (Takahashi and Kishima, 1973).

Discovery of a few methods have been identified to permit isolation and structure determination of natural products. The past few years have witnessed development in particular applications of novel separation technologies and modern spectroscopic techniques for structural analysis (Snyder et al., 1989). A column chromatography is a form of chromatography in which the stationary phase is retained in a column and generally used as a purification technique to isolate desired compounds from a mixture (Harvey, 2000). High-performance liquid chromatography (HPLC) method is better able to distinguish among different types of phenolics. While, nuclear magnetic resonance (NMR) spectroscopy has developed since the introduction of the first commercial spectrometers in the early 1960s and it is always a paramount important technique for use in structural analysis of natural products (Snyder, et al., 1989).

1.2 Current awareness and research problem statement

Various researches had been done to study the importance and properties of antioxidants from assorted natural sources yet still, to our best knowledge, there were small findings from some Malaysian timbers (Molyneux, 2003; Cruz et al., 2005; Kitzberger et al., 2007). Therefore besides other plant materials, findings of naturally occurring antioxidants from Malaysian timbers could contribute in replacing the synthetic antioxidants especially in foods, medicines and pharmaceutical products. More utilization of natural and safer antioxidants is desirable since synthetic antioxidant such as butylhydroxyanisole (BHA) have possible activity as promoters of carcinogenesis (Shimizu et al., 2002). Besides that, this study was to identify the ability of selected Malaysian commercial timbers to inhibit the growth of a brown rot and white rot fungi as the initial steps on developing environmental-friendly fungicides. This effort is hopefully would support other research in replacing traditional agrochemicals which has caused environmental pollution to our ecosystem (Quiroga et al., 2003; Yen et al., 2007)

1.3 Objectives

Malaysian commercial timbers are considered to be useful and some of them are notable as natural sources of numerous applications. The specific objectives of this study would be:

1. To investigate the antioxidant activities and total phenolic content of eleven Malaysian commercial timbers.
2. To identify the antifungal abilities of such timbers.
3. To purify and identify compounds from the most potential samples.

2.0 RESEARCH BACKGROUND

2.1 Malaysian commercial hardwood timbers

The forest areas of the whole continent of Asia fall into three groups. Some 42 per cent of the forests are coniferous and occur in the Himalayas and northwards, and in the Asia Minor, China and Japan. About 28 per cent are of temperate hardwoods or are mixed forests, and some occur in similar areas. While, the other 30 per cent of the forests are tropical hardwoods, occur south of Himalayas and many other countries (Brown, 1978). A report by Foxworthy (1927) stated that the area occupied by commercial forest with 2,500 known species in Peninsular Malaysia.

Commercial timbers are those that are regularly obtainable in the local market under their own names (Desch (a), 1957). Many species yield timbers with potential commercial significance and this fall broadly into four main categories; heavy hardwoods, medium hardwoods, light hardwoods and softwoods (Forest Department, 1968).

Two main classes of commercial timbers, the softwoods and hardwoods, are not classified based on their weight or hardness of the wood concerned, but it is based solely on its structure (Titmuss, 1971). Hardwoods possess more complex wood than conifers, having very short fibers and large diameter vessel elements, longitudinal parenchyma, and rays with differing types of cells. The hardwoods have the more typical flat, often broad leaf shape (Zobel and Buijtenen, 1989). Generally, commercial hardwood timbers are provided by dicotyledon, and those growing in temperate and cold regions are mostly deciduous. Most hardwood species are evergreen in warmer and tropical regions (Patterson, 1988). In the hardwoods, different types of cells do the separate work of conducting food, giving structural rigidity, and storing food (Titmuss, 1971).

2.2 Selected timbers – Family and species

2.2.1 Dipterocarpaceae

The Dipterocarpaceae family consists of about 19 genera and probably over 400 species of trees and shrubs. The Malaysian Dipterocarpaceae are medium-sized or large trees, and their economic value depends on the production of useful timbers (Desch (a), 1957). Dipterocarpaceae, the most typical family of our jungle, are essentially tropical evergreen rainforest trees, biologically unsuited to live under less warm and uniformly moist conditions. In their natural habitat, they disappear on mountain slopes at an altitude of 3000-4000 ft, and high range form impassable barriers (Symington, 1974). The timbers include some suitable uses for heavy constructional work, carcassing and joinery. As for general utility purposes, selected material of several species is employed in the manufacture of inexpensive furniture, and none can be classified as high-class furniture or special-purpose woods for interior fittings (Desch (a), 1957).

Keruing latek (*Dipterocarpus apterus*) is categorized as medium hardwood timber. *Dipterocarpus apterus* is one of the most important timbers of Malaysia and common in parts of central Johor. It is usually inhabits low-lying, flat jungle and sometimes is found in the neighbourhood of swampy ground or land. The fruits can be boiled and eaten as vegetable (Symington, 1974). *Dipterocarpus* species is essentially a utility timber which is suitable for constructional work, flooring, and railway sleepers (Desch (a), 1957). Another Dipterocarpaceae family member, Meranti seraya (*Shorea curtisii*) or also known as dark red meranti is classed as a light hardwood timber. It is moderately durable and commonly used as joinery, furniture, panelling, flooring, truck bodywork, plywood, and many more uses (FRIM, 1981). Goh et al. (1993) recorded that saponin was detected in the bark and wood of Meranti seraya. Beside that, alkaloids and terpene were found in the bark, wood and leaves.

2.2.2 Leguminosae

The Leguminosae is the second largest family of flowering plants, consisting of about 550 genera and between 12 000 to 15 000 species of trees, shrubs, climbers, and herbaceous plants. The family contains many valuable ornamental timbers which exhibit wide variation in colour, weight, hardness, grain, and texture. The seeds are rich in starch and proteins that are used for human consumption such as broad beans (*Vicia faba* L.), peas (*Pisum satirum* L.), ground nuts (*Lens esculentus* L.), soybean (*Glycine hispida* Maxim) and lentils clover (*Trifolium pretense* L. and *T. repens* L.). Several tree species are planted for their beautiful flowers (Desch (a), 1957).

Angsana or sena trees (*Angsana*), commonly known as 'narra' in Philipines, 'pradoo' in Thailand and 'sonokembang' in Indonesia, were noted growing in Malacca as early 1778. Angsana is a common wayside tree that can grow well within a short period of time (Nureza, 2004). Angsana is a light hardwood timber, with an average air-dry density of 625 kg/m^3 (39 lb /ft.^{3}). The sapwood of the timber is white or pale-straw, whereas the heartwood is golden brown or brown with darker coloured streaks (Sim, 1988). Takeuchi et al., (1985) reported that a nondialyzable polyphenolic substance having antiplasmin activity was isolated from the bark of Angsana. This plant has also shown antibacterial activity (Khan and Omoloso, 2003) and antifungal activity (Kusuma et al., 2005). Its red sap and bark has been used as remedies or native medicine against diarrhea and sprue, and the young leaves are used as maturative in Indonesia (Endo and

Miyazaki, 1972). Angsana is one of the best fine-furniture woods in Malaysia, and is used for furniture making, cabinets, interior trimming, and novelty items (Sim, 1988).

Another species in this research belonging to Leguminosae family is Kekatong (*Cynometra inaequifolia*). This heavy hardwood timber is relatively hard and heavy, averaging 1010 kg/m^3 (63 lb/ft^3) at 19% moisture content. The sapwood is lighter in colour and the heartwood is dark red in colour with brown-black streaks. Usually, the timber is used for heavy and medium construction, poles, beams, door and window frames, heavy duty flooring and sometimes for parquet flooring (Mohd.Shukari, 1983).

2.2.3 Apocynaceae

In tropical regions, the Apocynaceae consist of about 130 genera and considerably more than 1000 species of perennial herbs, shrubs, climbers, and trees. Certain species are important as timber producers, and the wood of some Apocynaceae members, however, is suitable for special purposes, such as turnery and fine carving. The timbers were reported to be the lightest woods in the world (Desch (a), 1957).

Jelutong (*Dyera costulata*) trees from Apocynaceae family grow to very large trees with straight cylindrical stems without buttresses and their latex has a constituent of chewing gum. Jelutong is categorized under light hardwood timbers and usually cut into boards for use in interior construction where strength is not an important criterion. However, it is more suitable for special purposes such as drawing boards, black boards, wooden models, etc. It is particularly good wood for pattern making and has proved successfully for battery separators (Thomas (a), 1970). Phytochemical report by Goh et al. (1993) stated that there was a minor amount of alkaloid in the leaves and stem. Meanwhile, terpene and flavonoid were isolated from the leaves.

2.2.4 Clusiaceae

The Clusiaceae or Guttiferae consist of over 40 genera and approximately 1000 species of tree, shrubs, and a few herbs. Some species are useful for decorative purposes because of the showy flowers and foliage of some trees, some species yield valuable timbers and several species produce fruits, oils, and resins (Desch (a), 1957).

Bintangor bukit (*Calophyllum symingtonianum*) and bintangor daun karat (*Calophyllum rubiginosum*) are among 45 species of *Calophyllum* spp. found in Peninsular Malaysia. There are other vernacular names of bintangor including 'bakakol' or 'entangor' in Sarawak and 'penaga laut' in Peninsular Malaysia and Sabah. The tree occurs throughout the country from lowlands to upper montane forests and it is extremely abundant in a small area of North East Johor. The timber is a light hardwood timber with an average air-density of 640 kg/m^3 (40 lb/ft^3) (Abdul Rashid, 1984). The timber is suitable for light constructional purposes under cover such as flooring, ceilings, partitioning and paneling since it is moderately hard and heavy and not durable under exposed conditions (Menon, 1960). It is also suitable for joinery and furniture. Besides that, bintangor is widely used in boat building especially spars and masts (Abdul Rashid, 1984).

Merawan siput jantan (*Hopea odorata*) is one of the *Hopea* groups, which represented in Malaysia by thirty species. Merawan are reported to be a moderately durable to durable wood when in contact with the ground in the tropics. It is expected to have an average life of about 5 years in severe conditions. This light hardwood timber is suitable for light and medium heavy constructional work because of its durability, except in direct contact with termite infested ground. Although it has no particularly attractive figure, merawan can be used for joinery and even for cabinet work because it worked with comparative ease and small shrinkage (Thomas (b), 1964).

2.2.5 Anacardiaceae

The Anacardiaceae are most abundantly distributed in warm or tropical regions and consist of about 60 genera and 500 species of trees, shrubs, and woody climbers, widely occurring throughout the world. There are about eighteen indigenous and exotic genera of the Anacardiaceae in the Peninsular Malaysia, including at least 80 distinct species. A few of those species are quite common in the coastal-swamp forests and others occur in the lowland of dipterocarp forest with a few are abundant in the hills. Some of the members of this family are producing general utility woods of local commercial value, a few have highly ornamental timber, and some have the soft, non-durable timber of little or no economic value. Some species are trees attaining considerable size and several yield good sawmill logs (Desch (a), 1957).

In this research work, merpauh periang (*Swintonia schwenkii*), which belongs to this family, was used. It is a medium hardwood timber and the wood is moderately hard and heavy. Generally, the sapwood and heartwood of *Swintonia* spp. are not differentiated in the material examined, although the outer part of wood is rather lighter in colour than the interior (Desch (a), 1957).

2.2.6 Meliaceae

The family consists of 45 genera and more than 1000 species of trees or shrubs, with a very few sub-herbaceous species. The Meliaceae is an important source of valuable commercial timbers because they have been known to be used in the furniture and cabinet trades for high-class joinery, paneling, and shop fittings, and sometimes as printers' blocks. They are widely grown throughout the tropical regions of the world, but particularly in the tropical or sub-tropical regions of America, Africa, and Asia, with a few species extending to the east coast of Australia and to New Zealand (Desch (b), 1954).

Sandoricum koetjape or its commercial name, Sentul, is a member of Meliaceae, native to India and Malaysia and is now widely distributed in Southeast Asia. For the record, plants in the Meliaceae are rich sources of a variety of sesquiterpenoids and triterpenoids (Intan et al., 2003) and there are proven isolated classes of constituents from Sentul from previous reports. Triterpenoids were isolated from the heartwood and bark of Sentul (King and Morgan, 1960). Bryonomic acid, bryonolic acid, mesoinositol and dimethyl mucate were found from its fruit hulls (Sim and Lee, 1972), while limonoids were reported isolated from the seeds (Kosela et al., 1995). The timber is said to be used for carts and boats in Myanmar, house building in Indonesia and clogs in Malaysia (Desch (b), 1954).

2.2.7 Euphorbiaceae

The species belonging to this family are widely distributed throughout the world but most numerous in the tropics. They consist of about 250 genera and upwards of 5000 species of trees, shrubs, and herbs. Desch (a) (1957) states that the family is represented in the Peninsular by over 70 genera and more than 300 species of small trees or shrubs, but about 14 genera contain tree species attaining timber size. They can be found in all types of primary and secondary jungle throughout the Peninsular. Some species are used for firewood and poles, and a few are converted from time to time in local sawmills. Some of the Euphorbiaceae are popular as poles for rafters and one species, *Elateriospermum tapos* Bl., is preferred for firewood, but the only species used industrially in the Peninsular is sesendok (*Endorspermum malaccense* Muell,-Arg.) for match stick and battens for plywood chests.

Pimeleodendron griffithianum or its common vernacular name, Perah ikan, is a small medium-sized tree of primary hill jungle and it is not of economic importance. The sapwood and heartwood are not differentiated and this timber would appear not to be durable. It is soft and light to moderately heavy (Desch (a), 1957).

2.3 Bark, heartwood, and sapwood

The outer layer of the trunk, branches or roots, which can be separated from the wood, is known to be the bark (Corner, 1951). The bark consists of all tissues of a mature stem or root outside of the vascular cambium. Its cells produced by the cork cambium and are important component of the protective material (Freeman, 2005). A special feature of the bark is the lenticels and they are air-holes which enable air to diffuse through the bark, the corkiness of which makes it imperious to air and water. Thus, they ventilate the living tissues of the interior of the tree (Corner, 1951).

Anatomically, wood is gradually build from secondary xylem, which as it ages, its cells filled with gums and resins, and causes the oldest portions of secondary xylem to become darker. This darker and denser wood nearer the central regions of a given trunk is called heartwood (Johnson, 2003). It is a core of dark, dead tissue and the formation of heartwood is a unique process in nature, in which the death of large portion of an organism is beneficial to that organism. The central core of the trunk is used for support, and the core is just as strong dead as alive and it is an obvious advantage to the tree. Due to its natural strength, the heartwood develops the defense mechanism to ensure it does not become infected under normal conditions (Hart, 1989).

The wood nearer the vascular cambium is called sapwood and it is still actively involved in transport within the plant (Johnson, 2003). It is a part of the tree consist a portion of the tissue remains alive and is in communication with living tissues in the bark, meanwhile participating in the vital activities of the organism (Brown et al., 1949). The bark, hardwood and sapwood are illustrated in Figure 2.1 and 2.2.

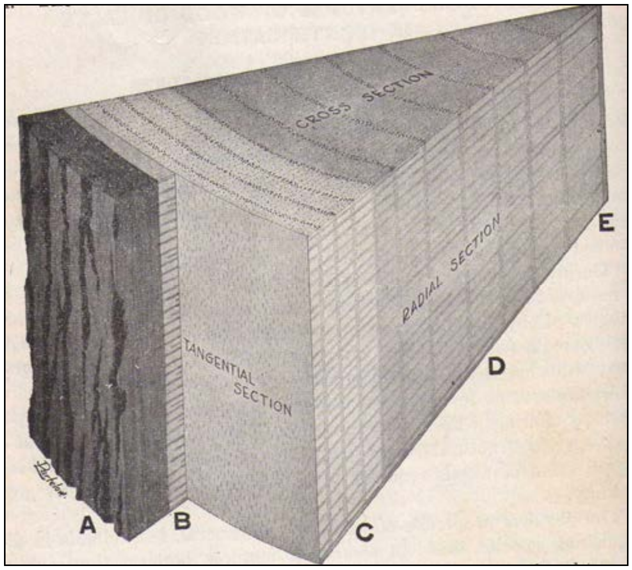

Figure 2.1. Parts of bark and wood. (A) Outer bark, (B) Inner bark, (C) Sapwood, (D) heartwood, (E) Pith (Source: Brown et al., 1949)

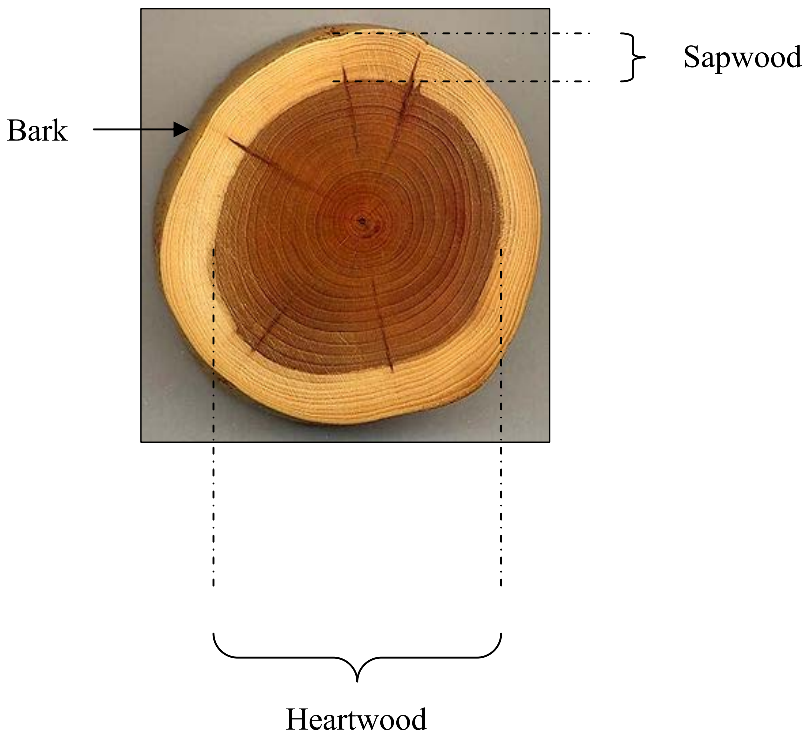

Figure 2.2. Bark, sapwood and heartwood (Source: Anonymous [a], 2008)

2.4 Extractives

Extractives are a large variety of wood components, representing a minor fraction and soluble in neutral organic solvents or water. The extractives comprise a large number of individual compounds of both lipophilic and hydrophilic types. The extractives can be regarded as nonstructural wood constituents, almost exclusively composed of extracellular and low-molecular-weight compounds. They occupy certain morphological sites in the wood structure. For example, the resin acids are located in the resin canals, whereas the fats and waxes are in the ray parenchyma cells. Phenolic extractives are present mainly in the heartwood and in the bark (Sjöström, 1993).

The content and composition of extractives vary among wood species. But, there are also variations depending on the geographical site and the season. Several woods contain extractable substances which are toxic or deterrent for bacteria, fungi and termites. Other extractives give colour and odour to the wood (Dietrich, 1931).

The content of extractives is higher in bark than in wood. It depends not only on the species but also on the solvents used. The diversity of the extractable compounds usually requires a sequential extraction, the yields from which give a preliminary characterization of the composition. The variation of the composition can be very large even within one genus. The alkali extract as well as the ethanol extract contain higher polymer flavonoids including the polyphenolic acids (Dietrich, 1931).

Different types of extractives are necessary to maintain the diversified biological functions of the tree. Fats constitute the energy source of the wood cells, whereas lower terpenoids, resin acids, and phenolic substances protect the wood against microbiological damage or insects attacks. The extractives are important not only for understanding the taxonomy and biochemistry of the trees, but also when considering technological aspects and constitute a valuable raw material for making organic chemicals (Sjöström, 1993).

Extractives also have significant influence on how wood is used. The studied chemistry, physics, structural dynamics, and commonly the amounts of extractives have been important information used for construction and goods since ancient time (Imamura, 1989).

2.4.1 Extraction and fractionation

Extraction of wood with organic solvents removes a broad spectrum of compounds including fatty acids, their esters, associated neutral materials, and some phenolic substances. The extractives components which contain fatty acids represent a major portion of the extractives in many woods. In the deciduous woods, the resin acids are minor constituents or are entirely absent (Browning, 1967).

For the isolation from wood or cellulose of substances soluble in selected solvents, any of several extraction devices may be applied. The most common is the Soxhlet extractor, which is highly efficient because of alternate siphoning and filling of the extractor body with fresh solvents. Soxhlet extractors are available in a variety of sizes. The larger ones accommodate several hundred grams of materials. Continuous extractors are less used due to the lower effectiveness of the extraction. The material to be extracted may be placed in a tall extraction thimble of alundum or of glass with a fritted-glass disk. Paper extraction thimbles are satisfactory unless fairly complete recovery of the extracted material is desired. In use of the larger extractors, the material may be placed in cloth bag, or the entire extractor body may be filled and the solvent exit covered with a loose plug of glass wool (Browning, 1967).

The ordinary organic solvents are available in a number of grades. Designations of purity are not uniform, and only a limited number of standards have been established. Specifications for reagent grades have been given by the American Chemical Society, together with appropriate methods of testing for impurities. Many solvents purified to meet a specific requirement, e.g., spectral or chromatographic purity. The best method of extraction depends on the sources. The usual solvents are water, alcohol, acetone, ether, and petroleum ether. Fresh or quickly dried materials must be used since enzymatic changes take place rapidly in a moist sample (Browning, 1967).

Fractionation is an important tool in the characterization of quantitative analysis of samples tested. It is used as well in the preparation of samples for further physical chemical measurements, identification and in direct determination of molecular weight and composition distribution in copolymers. Fractionation needs proper methods and purposes, whether in pure quantitative research or in quality control laboratories. In the earliest days of quantitative polymer science, the basic method for elucidating molecular weight distribution is by fractionation in different solubility. By the time passes, the major concern of fractionation has grown much interest in chemical analysis in the fields it does needed (Tung, 1977).

2.4.2 Chemical constituents and primary metabolites in plants

The main chemical constituents in the woody cell are cellulose, hemicelluloses, and lignin, which fill in about 95%. Cellulose is known as a long-chain polymer of glucose units bonded together with β-1,4 glycosidic bonds. Hemicelluloses are built of relatively short heterogeneous polymers of glucose, galactose, arabinose, mannose, xylose, and uronic acid units, linked by glycosidic bonds between the sugar residues. While, lignin is a complex polymer of phenylpropane units with many cross linkages and at least some covalent bonds exist between lignin and hemicelluloses. Some of the remainder percents can be considered extractives, which give the wood its colour, odour and also some physical properties (Hart, 1989).

Primary metabolites are those substances that are fundamental chemical units of living plant cells, such as nucleic acids, proteins and polysaccharides (Freeman, 2005). They are also the cytochromes, chlorophylls, and metabolic intermediates which occur in all plants and they all work together to suit the particular metabolic functions such as photosynthesis, respiration and physiological development (Heldt, 2005).

The whole field of natural products chemistry has been undergoing emerging process and positive progress in recent years and they do cover a large territory including bio-organic and bio-chemistry. The development of technology of isolation, purification, structure determination, and many more have reached an outstanding achievement among natural products researches. Thus, there is no doubt that the future in this area will become increasingly interdisciplinary and important (Nakanishi, 1989).

2.4.3 Secondary metabolites in plants

Secondary metabolites are often defined as structures and compositions that are closely related to substances that are required for basic cell activities (Freeman, 2005). Phenols, terpenes, alkaloids, fats, tannins, sugars, suberins, and caretenoids are classes of compounds known as secondary metabolites. In wood processing and utilization, the definition of secondary metabolites are everything that is not a structural polysaccharide or lignin and often referred to as 'extraneous components' because they are mostly extraneous to the lignocellulosic cell wall and are concentrated in resin canals and cell lumina, especially those of ray parenchyma cells. However, these types of compounds are actually found in all morphological regions and this definition cannot be strictly applied (Obst, 1998)

While such a definition emphasizing the structural components of wood is very functional, it can give the impression of demeaning the role of these 'extraneous components'. For example, if polysaccharides and lignins are the bones and flesh of woody tissue, it is the secondary metabolites that give woody plants their blood, soul and character. These components provide woods with their many colours and hues, scents and beauty (Gottlieb, 1990).

It has been commonly taught that while animals have developed efficient systems to excrete unwanted by-products, plants have few such mechanisms and usually must alter and then store their wastes ('secondary metabolites') (Manitto, 1981). For example, heartwood extractives retard wood decay, resin formation protects wounded tissues and toxic compounds in foliage and bark minimize insect and animal browsing through their poisonous properties (Gottlieb, 1990).

Varying by species, woods may contain as little as one percent or as much as one-third of their weights as secondary metabolites. Tropical and sub-tropical species typically contain greater amounts of extractives than do temperate zone woods. The concentration of secondary metabolites in trees is not uniform; generally higher amounts occur in bark, heartwood, roots, branch bases and wound tissues. Variations also occur among species, from tree to tree, and from season to season. Most of the special products may be extracted with neutral solvents, and such extracts are sometimes the source of useful materials (Obst, 1998).

2.4.3.1 Phenolic substances

The phenolic compounds are the most abundant classes of secondary metabolites found in plants and much concern has been given to their potential functional role in plant ecology. They comprise the bulk of the structural substances of plants and the pigmentation of flowers. Their functions include as defensive agents against herbivores and as signal molecules in plant interactions with pathogens, microbes, and parasitic angiosperms (Dalton, 1999). The major classes of plant phenolics include phenols, phenolic acids, quinones, xanthones, lignans and neolignans, stilbenes, flavonoids and tannins (Goodwin and Mercer, 1983).

The largest group of plant phenolics and the most studied is flavonoids which include more than 4000 different compounds. They are responsible for two-thirds of the phenolic in our diet, with the remaining one-third is from phenolic acids. Also known as the colour of health, flavonoids are polyphenolic compounds with some common characteristics that are widely found in fruits and vegetables. They might be important to biological function and health because studies in recent years had indicated that they had strong antioxidant activity. The members in flavonoids are flavones, flavonols, catechins, anthocyanidins, dihydroflavonols, isoflavones and many more (Ang, 2007).

Even though usually present in wood in relatively small quantities, flavonoids which can be found in both hardwoods and softwoods, may be importance in vivo in protecting the heartwood from decay, along with other phenolics that may be present. For example, dihydroquercetin, is one of the most potent, fungicidal materials of Douglas-fir, a fairly decay-resistant spesies. Flavonoids in vitro may contribute to the long-term durability and as a result, these compounds are usually of comparatively little biological or economic significance (Harborne, 1989).

Lignans is another group in plant phenolics and was introduced in 1936 as a group of naturally occurring phenylpropanoid dimers (Goodwin and Mercer, 1983). Some examples of lignans members are pinoresinol, lariciresinol, liovil, α-conodendrin, syringeresinol, and thomasic acid. Lignans, and their glycosides, are found in varying proportions in bark, wood, leaves, roots, fruits and seeds. Heartwood is much richer source of lignans than is in sapwood. In some cases, lignans are not present in all tissues, but may happen, for example, in heartwood but not elsewhere. Trees that are wounded, through physical injury or insect attack, often produce resins rich in lignans (neolignans or norlignans). Neolignans are also defined on a structural basis which they result from the coupling of polyphenols linked at positions other than the beta-beta coupling of the side chains. Although there is great opportunity for the formation of more varied and complex structures for neolignans, their occurrence is not widespread. Norlignans and norneolignans are structurally similar to lignans but have one less carbon atom and are C_{17} structures (Obst, 1998).

Meanwhile, tannin was described in 1976 as a group in plant phenolics which the materials were use to produce leather from animal skins. Because a wide variety of phenolics can act as tanning agents, more accurate definition have been employed. As a result, tannins have been separated into two classes based on their chemistry and origin, namely hydrolysable tannins and condensed tannins (Porter, 1989). The proanthocyanidins are the most commonly found class of tannins and are composed of over a hundred different oligomeric and polymeric structures (Hemingway and Porter, 1985). This class of compounds is proposed to be involved in plant defense mechanisms (Stafford, 1988), like many of the secondary metabolites.

2.4.3.2 Terpenes

The most widespread and chemically diverse groups of natural products from plants are the terpenes. They are a unique group of hydrocarbon-based natural products and further classified into hemiterpene, monoterpene, sesquiterpene, diterpene, sesterterpene, triterpene and tetraterpene. The terpenes are functioning in plants both ecological and physiological. Some of them restrain the growth of competing plants, many are known to be insecticidal and others are believed to attract insect pollinators (Brielmann, 1999).

Some compounds, such as chlorophyll pigments that have a diterpene side chain or gibberellins, are essential for the growth and well-being of plants. The terpenes constitute important components of many wood extractives and are often the major constituents of extracts obtained with nonpolar solvents. The most common terpenes from plants are essential oils, latexes and resinous exudates. Terpeness are found to occur both in free and combined states in nature and they are not highly functionalized occur free. The percentage and nature of terpenes in a given plant may vary widely with the time of harvesting of plant material and different parts of the same plant may contain different terpenes (Dev, 1989).

2.4.3.3 Alkaloids

Another large group of secondary metabolites isolated from the plants are alkaloids. There are more than 10 000 different alkaloids have been discovered in species from over 300 plant families (Anonymous (b), 1998). Some of the alkaloids have been known for hundred years such as cinchona alkaloids which has been known since 1639 as an antimalarial agent. They are present in the bark of *Cinchona* spp. and *Remijia* spp. plants indigenous to the high eastern slopes of the Andes. Usually alkaloids contain one or more of carbon atoms, all with nitrogen atom in the ring (Goodwin and Mercer, 1983). Some alkaloids have extraordinary structural similarities with neurotransmitters in the central nervous system of humans, including dopamine and serotonin. The great effect of these alkaloids on humans has led to the development of powerful pain-killer medications and spiritual drugs which also can cause serious addictions by people who are ignorant of the properties of these powerful chemicals (Anonymous (b), 1998).

2.4.3.4 Other metabolites

There are thousands of substances known and identified as secondary plant metabolites and all exhibits an impressive range of organic compounds that obviously provide a selective advantage to the plants themselves. Majority of the secondary metabolites, such